Olfactory Bulb Short Axon Cell Release of GABA and Dopamine Produces a Temporally Biphasic Inhibition–Excitation Response in External Tufted Cells

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Evidence for coexpression of two or more classic neurotransmitters in neurons has increased, but less is known about cotransmission. Ventral tegmental area (VTA) neurons corelease dopamine (DA), the excitatory transmitter glutamate, and the inhibitory transmitter GABA onto target cells in the striatum. Olfactory bulb (OB) short axon cells (SACs) form interglomerular connections and coexpress markers for DA and GABA. Using an optogenetic approach, we provide evidence that mouse OB SACs release both GABA and DA onto external tufted cells (ETCs) in other glomeruli. Optical activation of channelrhodopsin specifically expressed in DAergic SACs produced a GABA_A receptor-mediated monosynaptic inhibitory response, followed by DA–D₁-like receptor-mediated excitatory response in ETCs. The GABA_A receptor-mediated hyperpolarization activates I_h current in ETCs; synaptically released DA increases I_h , which enhances postinhibitory rebound spiking. Thus, the opposing actions of synaptically released GABA and DA are functionally integrated by I_h to generate an inhibition-to-excitation “switch” in ETCs. Consistent with the established role of I_h in ETC burst firing, we show that endogenous DA release increases ETC spontaneous bursting frequency. ETCs transmit sensory signals to mitral/tufted output neurons and drive intraglomerular inhibition to shape glomerulus output to downstream olfactory networks. GABA and DA cotransmission from SACs to ETCs may play a key role in regulating output coding across the glomerular array.

Introduction

Dopamine (DA) plays important roles in motor behaviors, Parkinson's disease, reward reinforcement, addiction, working memory, and schizophrenia (Greengard, 2001; Björklund and Dunnett, 2007; Iversen and Iversen, 2007; Beaulieu and Gainetdinov, 2011). This modulatory neurotransmitter is distributed in nine major neuron groups, including the substantia nigra, ventral tegmental area (VTA), and olfactory bulb (OB) (Dahlstroem and Fuxe, 1964; Björklund and Dunnett, 2007).

DA influences odor discrimination (Kruzich and Grandy, 2004; Yue et al., 2004; Pavlis et al., 2006; Tillerson et al., 2006; Wei et al., 2006; Doty, 2012). D₂-like receptors are predominantly present in olfactory nerve (ON) terminals and glomerular layer (GL); D₁-like receptors are widely distributed throughout OB, except for the olfactory nerve layer (ONL) (Nickell et al., 1991; Coronas et al., 1997; Koster et al., 1999; Gutiérrez-Mecinas et al., 2005). DA presynaptically inhibits the first synapse of the olfactory system by reducing glutamate release from ON terminals via

D₂-like receptors (Wachowiak and Cohen, 1999; Berkowicz and Trombley, 2000; Ennis et al., 2001).

In the OB tyrosine hydroxylase (TH), the rate-limiting enzyme in DA biosynthesis, is localized to neurons predominantly within the GL (Hököfelt et al., 1975; Baker et al., 1983; Kosaka et al., 1985; Gall et al., 1987; McLean and Shipley, 1988; Goheen Robillard et al., 1997). The vast majority of juxtaglomerular cells (JGCs), a heterogeneous population of interneurons surrounding each glomerulus, are GABAergic (Parrish-Aungst et al., 2007; Kiyokage et al., 2010). In contrast to VTA TH-expressing (TH⁺) neurons that corelease DA, glutamate, and GABA (Chuhma et al., 2004; Hnasko et al., 2010; Stuber et al., 2010; Tecuapetla et al., 2010; Yamaguchi et al., 2011; Tritsch et al., 2012), TH in JGCs colocalizes with GABA and glutamic acid decarboxylase (GAD), the rate-limiting enzyme for GABA biosynthesis (Hököfelt et al., 1975; Baker et al., 1983; Kosaka et al., 1985; Gall et al., 1987; Goheen Robillard et al., 1997; Kosaka and Kosaka, 2008; Kiyokage et al., 2010). This suggests that DA and GABA are cotransmitters in OB. Consistent with this idea, GABA mediates self-inhibition in TH⁺ JGCs (Maher and Westbrook, 2008).

JGCs coexpressing markers for DA and GABA are short axon cells (SACs), which send extensive processes to multiple neighboring glomeruli, forming the interglomerular circuit (IGC) (Kosaka and Kosaka, 2008; Kiyokage et al., 2010). These observations raise several questions. (1) Do SACs cotransmit GABA and DA? (2) What are the effects of GABA–DA cotransmission on postsynaptic targets? (3) What is the impact of cotransmission at the circuit level? To address these questions, we investigated synaptic transmission from SACs to external tufted cells (ETCs), a

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key glomerular neuron that gates the glomerular output by transferring ON input to their postsynaptic targets, including the majority of GABAergic periglomerular cells (PGC), GABA/DAergic SACs (Hayar et al., 2004a; Shao et al., 2009; Kiyokage et al., 2010), and the main output neurons of the OB—mitral/tufted cells (M/TCs) (De Saint Jan et al., 2009; Gire et al., 2012). To ensure specific activation of SACs, we used viral expression of cre-dependent channelrhodopsin 2 (ChR2) in a line of transgenic mice in which the TH promoter drives *cre*. Our previous study showed that all TH⁺ SACs coexpress GAD-67 (Kiyokage et al., 2010); thus, this optogenetic approach provided us a precise tool to investigate GABA–DA cotransmission through selective activation of SACs.

Materials and Methods

Animals. Wild-type male mice (C57BL/6J) and transgenic TH–Cre mice [B6.Cg–Tg(TH–Cre)1Tmd/J] were obtained from Charles River and The Jackson Laboratory, respectively. A colony of transgenic animals was maintained by breeding heterozygous male TH–Cre mice with wild-type C57BL/6J female mice. Animals were maintained with a standard 12 h light/dark cycle and given food and water *ad libitum*. All experimental procedures were performed in accordance with protocols submitted to and approved by the University of Maryland Institutional Animal Care and Use Committee.

ChR2 expression. Adeno-associated virus serotype 9 (AAV2.9) carrying fusion genes for ChR2 and enhanced yellow (EYFP) fluorescent protein or mCherry (Tsai et al., 2009) (University of Pennsylvania Vector Core, Philadelphia, PA) were injected into the GL of the medial side of each OB between postnatal weeks 4 and 6. Under deep anesthesia, skull was exposed, and a small hole was drilled over each OB with typical coordinates at 3.95 mm from bregma and 0.2 mm from midline. AAV2.9 was injected into three points within the GL of the medial side of each bulb (depth of 2.0, 1.5, and 1.0 mm) at a rate of 0.1 μ l/min for 5 min. After 2–3 weeks for ChR2–EYFP fluorescent protein expression, acute OB slices were prepared for experiments.

Slice preparation. Acute OB slices from 6- to 8-week-old male mice were prepared as described previously (Liu and Shipley, 2008a). Briefly, horizontal slices (350 μ m) were cut with a VT1200s Vibratome in an ice-cold and oxygenated (95% O₂–5% CO₂) sucrose-based artificial CSF (ACSF) containing the following (in mM): 204.5 sucrose, 3 KCl, 4.5 N, N-bis(hydroxyethyl)-2-aminoethane-sulfonic acid (BES), 2.6 MgSO₄, 0.5 CaCl₂, 26 NaHCO₃, and 10 glucose. After 30 min incubation in normal ACSF at 30°C, slices were then transferred to ACSF at room temperature until they were used for experiments. Normal ACSF was continuously bubbled with 95% O₂–5% CO₂ and had the following composition (in mM): 124 NaCl, 3 KCl, 4.5 BES, 1.3 MgSO₄, 1.3 CaCl₂, 26 NaHCO₃, and 10 glucose. During experiments, slices were perfused at 3 ml/min with ACSF equilibrated with 95% O₂–5% CO₂ and warmed to 30°C.

Electrophysiology. Whole-cell patch-clamp or cell-attached recordings were made from OB TH–EYFP-labeled SACs and ETCs visualized using BX50WI (Olympus) fixed-stage upright microscope equipped with near-infrared differential interference contrast (DIC) optics. SACs were identified by their expression of EYFP. ETCs were identified as described previously (Liu et al., 2012). Briefly, they are identified for experiments based on the following three criteria: (1) spontaneous intrinsic burst firing that persists even when fast synaptic transmitter (AMPA, NMDA, and GABA_A) receptors are blocked; (2) “pear”-shaped cell body located in the deep half of the GL when viewed in infrared DIC optics; and (3) an apical dendrite with extensively ramified tuft confined to the glomerulus and absence of lateral dendrites in the external plexiform layer.

Current or voltage signals were recorded with a MultiClamp 700B amplifier (Molecular Devices), low-pass filtered at 4 kHz, and sampled at 10 kHz with a DIGIDATA 1322A 16-bit analog-to-digital converter (Molecular Devices) using Clampex 9.2 (Molecular Devices). Patch recording electrodes were pulled from standard-wall glass capillary tubes without filament (Sutter Instruments). Patch pipettes (4–7 M Ω) for whole-cell recording contained the following (in mM): 115 K-gluconate,

5.0 EGTA, 0.63 CaCl₂, 5.5 Mg-Cl₂, 10 HEPES, 3 Na₂-ATP, 0.3 Na₃-GTP, and 14 Tris-phosphocreatine, pH 7.3 (285–295 mOsm). The liquid junction potential (11–13 mV) was not corrected. Access resistance was typically <30 M Ω and not compensated.

Electrical and optical stimulation. Electrical stimulation was delivered by bipolar glass electrodes made from theta borosilicate tubes (Sutter Instruments). The isolated and constant-current stimulation pulses (100 μ s) were triggered by a PG4000A digital stimulator (Cygnus Technology). Optical stimuli were delivered from a 25 μ m multimode optical fiber (0.1 numerical aperture, $\sim$ 7° beam spread; Thorlabs) coupled to a 150 mW, 473 nm, diode-pumped, solid-state laser (LWBL473083272; Made-in-China) and gated with a Uniblitz shutter. Optical power delivered at the fiber tip was calibrated with a PM20A power meter (Thorlabs). Onset and duration of optical stimulation was measured during every experiment by splitting 1% of the laser beam out to a high speed (30 ns rise time) silicon photosensor (model 818-BB; Newport) and recorded by the same MultiClamp 700B amplifier as the patch electrode.

Data analyses. Data were analyzed with Clampfit 9.2 (Molecular Devices), SigmaPlot 9.0 (Systat Software), NeuroExplorer (Nex Technologies), and Origin 8.5 (OriginLab). All data were presented as mean $\pm$ SE. Statistical analyses were performed with NCSS 8.0 (NCSS Software). Statistical significance for group comparisons of repeated measures from the same neurons were determined by either paired Student's *t* tests (see Figs. 1C, 4E) or one-way repeated-measures ANOVA with Bonferroni's test (see Figs. 2H, 3F, G, 5E, 6E–H). Statistical significance of nonrepeated measures from different neurons were calculated with one-way ANOVA with Bonferroni's multiple-comparison test (see Figs. 4C, G, 5F, G) or nonpaired Student's *t* test (see Fig. 4C, DHX and SCH + DHX).

Immunohistochemistry. OB slices (350 μ m thickness) were fixed in 4% paraformaldehyde for 24 h after physiological recordings. Then they were embedded in 10% gelatin in 1 $\times$ PBS and recut using a vibratome (Leica) at 40 μ m. Immunohistochemistry was performed at room temperature on free-floating sections. After several washes, sections were incubated for 30 min in 2% bovine serum albumin in TBST (0.1 M Tris, pH 7.4, 0.9% saline, and 0.3% Triton X-100) and then incubated overnight with a mouse anti-TH antibody (1:10,000; 22941; Immunostar). Sections were rinsed and incubated in anti-mouse Cy5-conjugated secondary antibodies (1:1000; Jackson ImmunoResearch) for 2 h at room temperature. Biocytin was detected using an anti-streptavidin Cy3-conjugated antibody (1:1000; Jackson ImmunoResearch) for 2 h at room temperature. EYFP was detected by the intrinsic fluorescence of the molecule. Sections were washed and mounted on gelatin-coated slides and coverslipped with a 1,4-diazabicyclo-[2.2.2]octane-based anti-fade mounting media. Digital microscopy images were captured using a Fluoview500 confocal microscope (Olympus) and assembled using Corel-Draw X4 (Corel).

Drugs and chemicals. DL-2-Amino-5-phosphonovaleric acid (APV; 50 μ M), 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide disodium salt (NBQX disodium salt; 10 μ M), gabazine (GBZ; SR95531; 10 μ M), 4-ethylphenylamino-1,2-dimethyl-6-methylaminopyrimidinium chloride (ZD7288; 20 μ M), (±)-*trans*-10,11-dihydroxy-5,6,6a,7,8,12b-hexahydrobenzo[a]phenanthridine hydrochloride (DHX hydrochloride; 10 μ M), octahydro-12-(hydroxymethyl)-2-imino-5,9,7,10a-dimethano-10aH-[1,3]dioxocino[6,5-d]pyrimidine-4,7,10,11,12-pentol citrate (TTX citrate; 1 μ M), (6a*S-trans*)-11-chloro-6,6a,7,8,9,13b-hexahydro-7-methyl-5H-benzo[d]naphth[2,1-b]azepin-12-ol hydrobromide (SCH39166 hydrobromide; 10 μ M), and (2*S*)-3-[(1*S*)-1-(3,4-dichlorophenyl)ethyl]amino-2-hydroxypropyl(phenylmethyl)phosphinic acid (CGP55845; 10 μ M) were purchased from Tocris Cookson. 2-(3,4-Dihydroxyphenyl)ethylamine hydrochloride, 3,4-dihydroxyphenethylamine hydrochloride, 3-hydroxytyramine hydrochloride, 4-(2-aminoethyl)-1,2-benzenediol hydrochloride (dopamine hydrochloride; 20 μ M), (S)-5-aminosulfonyl-N-[(1-ethyl-2-pyrrolidinyl)methyl]-2-methoxybenzamide [(S)-(–)-sulpiride; 20 μ M], and all other chemicals were purchased from Sigma-Aldrich. All drugs were bath applied by diluting in ACSF at the above indicated doses unless otherwise stated.

Figure 1. Stimulation of the IGC produces an inhibitory monosynaptic response in ETCs. **A**, Schematic diagram showing the experimental design. **B**, Voltage-clamp recording traces showing the synaptic responses in ETCs (held at 0 mV) to interglomerular stimulation. Thick traces represent the average. **C**, Population data from five ETCs showing that the IPSC amplitude in the absence (control) or presence (10 μ M) of GBZ. *** p < 0.001 (n = 5 cells, paired Student's t test). **D**, Pooled data from five ETCs showing the IPSC latencies and their variation.

Results

SACs monosynaptically inhibit ETCs

SACs coexpress TH and GAD-67, a rate-limiting enzyme for GABA biosynthesis, suggesting that their synapses release both GABA and DA (Kiyokage et al., 2010). SACs have extensive processes that innervate many neighboring glomeruli, thus forming the IGC (Aungst et al., 2003; Kosaka and Kosaka, 2008; Kiyokage et al., 2010). The interglomerular postsynaptic targets of SACs are not known. One potential target is the ETC, a glomerular neuron that receives direct ON synapses and provides monosynaptic excitatory input to M/TCs and the majority of local PGCs and SACs. ETCs thus play a key role in both excitatory and inhibitory glomerular network operations (Hayar et al., 2004a; De Saint Jan et al., 2009; Gire and Schoppa, 2009; Shao et al., 2009; Kiyokage et al., 2010; Gire et al., 2012).

If SACs release GABA onto ETCs, then interglomerular stimulation should produce GABA-mediated monosynaptic responses in ETCs. To stimulate interglomerular axons in isolation from other intrabulbar connections, we prepared slices with two microsurgical cuts: one through the ONL and a second through all layers deep to the glomeruli leaving only interglomerular connections intact (Aungst et al., 2003). ETCs rostral to the cuts were voltage clamped at 0 mV to optimize detection of IPSCs while electrical stimulation was delivered to the GL caudal to the cuts (Fig. 1A). To further enhance detection of IPSCs and to minimize other potential circuit effects recruited by glomerular stimulation, NBQX (10 μ M) and APV (50 μ M) were present throughout the experiments. As predicted, interglomerular stimulation evoked an outward current that was abolished by GBZ (10 μ M; Fig. 1B), indicating mediation by GABA_A receptors. The peak amplitude of this response was 38.4 ± 4.2 pA (n = 5) in control

and 0.3 ± 0.1 pA (n = 5, p < 0.001) in the presence of GBZ (Fig. 1C). The short response latency (2.2 ± 0.3 ms, n = 5) and low-latency jitter (165 ± 17 μ s, n = 5) are consistent with monosynaptic GABAergic interglomerular transmission from SACs to ETCs (Fig. 1D).

With electrical stimulation, it is impossible to rule out that other unidentified glomerular neurons, in addition to SACs, contribute interglomerular monosynaptic input to ETCs. To selectively activate DA–GABAergic SACs, we devised a Chr2-based optogenetic strategy in which a cre-inducible AAV2.9 vector carrying an inverted double-floxed Chr2–EYFP fusion construct was microinjected into the GL of TH–Cre mice. This consistently yielded Chr2–EYFP expression that was restricted to the GL (Fig. 2A) in which DA neurons are predominantly distributed (Dahlstroem and Fuxe, 1964; Kosaka et al., 1985; Gall et al., 1987; Goheen Robillard et al., 1997; Björklund and Dunnett, 2007; Parrish-Aungst et al., 2007; Kiyokage et al., 2010). Immunohistochemical staining of TH protein (Fig. 2B,C) confirmed that $90.8 \pm 0.6\%$ (n = 108 cells from 3 mice) of Chr2⁺ glomerular neurons were TH⁺. The remaining ~10% may represent immature SACs that express the TH gene and drive Chr2 but do not yet express detectable levels of TH protein (Baker et al., 2001; Saino-Saito et al., 2004).

To characterize Chr2 channels and the efficacy of light activation in SACs, we made whole-cell patch-clamp recordings from Chr2⁺ cells. *Post hoc* colocalization of Chr2–EYFP, TH, and biocytin loaded into the recorded cells via the patch pipette confirmed that all recorded neurons were Chr2⁺ DAergic SACs (Fig. 2C). Chr2⁺ glomerular neurons exhibited typical electrophysiological properties of SACs with a mean membrane potential of -69.5 ± 2.1 mV and a mean resistance of 639.3 ± 9.7 M Ω (n = 7 cells), consistent with previously reported values for SACs in wild-type animals (Hayar et al., 2004a). This indicates that Chr2 expression does not influence the basic electrical properties of SACs. Next we investigated the responses of Chr2⁺ SACs to 473 nm laser light activation. To minimize exposure to laser light, which might cause phototoxicity (Hopt and Neher, 2001), exposure was limited to 3 ms in all the experiments. All tested cells showed a light-intensity-dependent inward current (Fig. 2D, n = 5 cells) when held at -70 mV in the presence of TTX (1 μ M) to eliminate action currents and circuit influences. The peak amplitude of this light-evoked current from five cells was 69.8 ± 18.5 pA at 7 μ W, 510.7 ± 114.2 pA at 520 μ W, and 1213.9 ± 175.8 pA at 3.75 mW of laser light (Fig. 2D). The insensitivity of the current to TTX and fast synaptic transmission blockers (10 μ M NBQX, 50 μ M APV, and 10 μ M GBZ) confirmed that it was attributable to direct activation of Chr2 in SACs.

In current clamp, laser light exposure evoked action potentials in Chr2⁺ SACs. The number of spikes increased (Fig. 2E) from 1.7 ± 0.7 , 4.6 ± 1.4 , to 5.6 ± 1.4 whereas the latency of the first spike decreased from 3.8 ± 0.5 , 1.3 ± 0.3 , to 0.9 ± 0.2 ms with incremental light intensity (Fig. 2F) from 7 μ W, 520 μ W, to 3.75 mW, respectively (n = 5 cells). Although 3.75 mW yielded the maximum number of spikes from SACs, repetitive exposure to light of this intensity could potentially cause phototoxicity (Hopt and Neher, 2001). Thus, in all subsequent experiments, we used 520 μ W, which was the lowest light intensity yielding robust near-maximal SAC spiking (~85% of maximum; Fig. 2E).

Next we investigated the SAC $\rightarrow$ ETC synaptic connection. ETCs were voltage clamped at -55 mV, and optical stimulation was applied to SACs, four to five glomeruli distant from the recorded ETC (Fig. 2G, top). Optical stimulation reliably evoked an outward current in ETCs (Fig. 2G,H). This outward synaptic

Figure 2. Activation of DAergic SACs generates an inhibitory monosynaptic response in ETCs in the IGCs with optogenetic approach. **A**, Confocal image of a horizontal OB section showing the expression of ChR2–YFP (green) selectively in the GL. **B**, Confocal images showing the colocalization of TH protein (blue, left) and ChR2–YFP (green, middle) in SACs. **C**, One recorded SACs labeled with three markers. TH protein (blue), ChR2–YFP (green), and biocytin (red) filled via a whole-cell patch electrode. **D–F**, Population data plotting the peak amplitude of ChR2 current (**D**, $n = 5$ cells), spikes numbers evoked each laser light exposure (**E**, $n = 5$ cells), or the first spike latency (**F**, $n = 5$ cells) against the power of laser light (3 ms duration) in SACs. Insets, **D**, Typical traces showing ChR2 currents evoked by laser light at increasing power; **E**, representative current-clamp response trace showing spikes evoked by laser light exposure at power level as indicated in the plotting graph (red); **F**, typical current-clamp recording traces showing the first spike latency decreases with incremental laser power. **G**, Top shows experimental design; bottom shows ETC synaptic currents in response to optical stimulation of SACs in distant glomeruli before (control) and after (10 μ M) GBZ. EPL, External plexiform layer. **H**, Population data showing that GBZ reversibly abolishes the outward synaptic current in ETCs ($n = 5$) evoked by stimulation of distant glomeruli. *** $p < 0.001$ ($n = 5$ cells, one-way repeated-measure ANOVA with Bonferroni's test).

current was reversibly abolished by 10 μ M GBZ (74.4 ± 7.2 pA in control vs 2.2 ± 0.5 pA in GBZ; $n = 5$, $p < 0.001$), indicating a GABA_A receptor-mediated IPSC triggered by optical stimulation of SACs. To estimate the latency of this optical stimulation-evoked IPSC, we measured the time from the onset of light to the onset of the IPSC and subtracted 1.3 ms, which is the average latency of the first spike elicited in SACs by 520 μ W optical stimulation. The average latency of optically evoked IPSCs in five ETCs was 2.15 ± 0.12 ms (range of 1.73–2.69 ms), and the jitter was 139.28 ± 14.25 μ s (range of 89.46–178.37 μ s; $n = 5$ cells). These values are consonant with the latencies measured with electrical stimulation and are consistent with monosynaptic transmission (Doyle and Andresen, 2001). Together, these exper-

iments indicate that SAC interglomerular synapses release GABA that produces monosynaptic GABA_A receptor-mediated inhibition in ETCs.

Interglomerular inhibition produces a rebound excitation in ETCs

How does SAC inhibitory input impact ETC output? ETCs generate spontaneous bursts of action potentials (Hayar et al., 2004b; Liu and Shipley, 2008a). Each burst contains two to five action potentials with different ETCs generating spontaneous bursts at different burst frequencies ranging from 0.15 to 11 Hz (mean, 3.3 ± 0.14 Hz; $n = 288$ cells) (Liu et al., 2012). Light activation of SACs in distant glomeruli reproducibly evoked IPSPs, which ter-

Figure 3. Optical stimulation of SACs generates a GABA_A receptor-mediated monosynaptic IPSP, followed by rebound excitation in ETCs. **A–C**, Top, Twenty current-clamp traces showing responses of the same ETC to optical stimulation of distant glomeruli in normal ACSF (**A**), ACSF containing 10 μ M NBQX and 50 μ M APV (**B**), and ACSF containing NBQX, APV, and 10 μ M GBZ (**C**). Middle, Raster plots showing spike distribution across 20 individual traces in corresponding conditions. Blue vertical lines indicate the blue laser light exposure. Bottom, Spike histogram averaged from eight ETCs (20 traces/cell) showing fast inhibition, followed by rebound excitation in response to optical stimulation of distant glomeruli in corresponding conditions shown in the top. **D**, Close-up of IPSPs evoked by optical stimulation of distant glomeruli within the purple rectangles shown in the corresponding top panels. Thick traces represent the average from 20 traces (gray). **E**, Population data showing the IPSP onset and peak latencies ($n = 8$ cells). **F**, **G**, Population data from eight cells showing the effects of NBQX + APV, or NBQX + APV plus GBZ on IPSP amplitude (**F**) and rebound spiking (**G**). *** $p < 0.001$ ($n = 8$ cells, one-way repeated-measure ANOVA with Bonferroni's test).

minated spontaneous bursting in ETCs (Fig. 3A). The optically evoked IPSP had an onset latency of 2.2 ± 0.1 ms and reached peak amplitude (8.1 ± 0.9 mV, $n = 8$ cells) at 10.1 ± 0.5 ms ($n = 8$ cells). Inhibition of spiking was invariably followed by a rebound spike burst (Fig. 3A). Neither the optically evoked IPSP (8.1 ± 0.9 mV in ACSF vs 8.2 ± 0.9 mV in NBQX + APV, $n = 8$, $p > 0.05$; Fig. 3D,F) nor the rebound spike burst ($195.0 \pm 9.7\%$ in ACSF vs $199.0 \pm 13.7\%$ in NBQX + APV, $n = 8$, $p > 0.05$; Fig. 3A,B,G) were altered by blocking fast glutamatergic transmission with NBQX (10 μ M) and APV (50 μ M; Fig. 3B). Thus, SAC $\rightarrow$ ETC inhibition–excitation sequence does not depend on excitatory elements. In contrast, GBZ (10 μ M) completely abolished both the IPSPs (Fig. 3C,D,F, 8.2 ± 0.9 mV in NBQX + APV vs 0.2 ± 0.1 mV in NBQX + APV plus GBZ; $n = 8$, $p < 0.001$) and the rebound burst (Fig. 3C,G, $199.0 \pm 13.7\%$ in NBQX + APV vs $103.8 \pm 1.9\%$ in NBQX + APV plus GBZ; $n = 8$, $p < 0.001$). Together, these data show that GABA release from SACs is necessary and sufficient for the inhibition–excitation response in ETCs.

DA produces an I_h -mediated inward current via D₁-like receptors in ETCs

Coexpression of TH suggests that SACs also release DA, but direct physiological evidence for this is lacking. If DA is synaptically released with GABA from SACs, what is its postsynaptic action in ETCs? To address this, we first examined the effect of exogenous DA to determine whether ETCs express DA receptors, which receptor types, and characterize the currents DA generates in ETCs. In the presence of ionotropic glutamate (iGluR) and GABA_A receptor blockers (10 μ M NBQX, 50 μ M APV, and 10 μ M GBZ) to minimize circuit effects, bath application of DA (20 μ M) reliably produced an apparent inward current with peak amplitude of 8.1 ± 1.5 pA ($n = 6$) in ETCs held at -55 mV (Fig. 4A,C). Application of the selective D₁-like receptor antagonist SCH39166 (10 μ M) blocked the DA (20 μ M)-induced inward current; a residual small outward current was present in four of

six cells (2.5 ± 0.6 pA, $n = 4$, $p < 0.05$, Kolmogorov–Smirnov test). Addition of the D₂R antagonist S-(–)-sulpiride (50 μ M; Fig. 4A,C) with the D₁R antagonist abolished this current (0.18 ± 0.11 pA, $n = 5$, $p < 0.001$). These results indicate that the predominant effect of DA is an inward current that is mediated by D₁-like receptors in all ETCs. In separate experiments the D₁-like receptor agonist DHX (10 μ M) produced an inward current (14.5 ± 3.2 pA, $n = 5$, $p > 0.05$ compared with the DA-induced inward current of 8.1 ± 1.5 pA, $n = 6$); the DHX inward current was completely blocked by the selective D₁-like receptor antagonist SCH39166 (10 μ M, 0.6 ± 0.2 pA, $n = 5$, $p < 0.001$ compared with DHX alone; Fig. 4B,C). These results show that DA has a predominantly excitatory effect mediated by D₁-like receptors in all ETCs and a fourfold smaller inhibitory effect mediated by D₂-like receptors in some ETCs.

ETCs respond to exogenous DA, but do they respond to DA released from SACs? Optical stimulation of distant glomeruli induced an inward current (5.2 ± 1.0 pA, $n = 5$) in the presence of GBZ to eliminate the GABA-mediated IPSCs. This inward current was eliminated by a selective D₁-like receptor antagonist (SCH39166 at 10 μ M, $n = 5$, $p < 0.001$; Fig. 4D,E). This shows that SACs release both DA and GABA onto ETCs generating a GABA_A receptor-mediated outward (hyperpolarizing) current and DA–D₁-like receptor-mediated inward (depolarizing) current.

D₁-like receptors are G-protein-coupled metabotropic receptors whose downstream transduction pathways target multiple membrane conductances, including sodium currents, hyperpolarization-activated cation current, calcium currents, and potassium currents (Schiffmann et al., 1998; Zhang et al., 1998, 2002; Dong and White, 2003; Chen and Yang, 2007; Kisilevsky et al., 2008; So et al., 2009; Ballo et al., 2010). Many of these conductances regulate ETC spontaneous bursting (Liu and Shipley, 2008a). To determine which ETC conductances are modulated by D₁-like receptor activation, we first determined whether the effects of DA on ETCs were persistent or transient. A

Figure 4. DA produces an apparent inward current by enhancing I_h in ETCs. **A**, Current traces showing effects of the selective D_1 -like receptor antagonist SCH39166 (SCH, $10 \mu\text{M}$, brown), the selective D_2 -like receptor antagonist (S)-(–)-sulpiride (sul., $50 \mu\text{M}$), or SCH39166 plus (S)-(–)-sulpiride (SCH&sul., red bar) on inward current induced by bath application of exogenous DA ($20 \mu\text{M}$, red bar). **B**, Current traces showing that the selective D_1 -like receptor agonist DHX ($10 \mu\text{M}$)-induced inward current is abolished by SCH39166 (brown). **C**, Population data from ETCs as shown in **A** and **B**. Red symbols represent DA experiments, and green symbols stand for DHX experiments. Symbol shapes represent different treatments. *** $p < 0.001$ (one-way ANOVA with Bonferroni's test, $n = 6$ cells in DA experiments and $n = 5$ cells in DHX experiments). **D**, Current traces showing response of the same ETC to optical stimulation (blue vertical bar) of a distant glomerulus before (black) and after ($10 \mu\text{M}$, brown) SCH39166. **E**, Population data from five ETCs showing that the optical activation of distant SACs evoked an apparent inward current that is significantly attenuated by SCH39166. *** $p < 0.001$ ($n = 5$ cells, paired Student's t test). **F**, Current traces showing ETC response to bath-applied DA ($20 \mu\text{M}$) in ACSF (contr.), with the addition of TTX ($1 \mu\text{M}$) or ZD7288 (ZD, $20 \mu\text{M}$). **G**, Population data showing the DA-induced inward current.

10 min perfusion of DA ($20 \mu\text{M}$) produced an apparent inward current that showed no inactivation throughout the application, indicating that DA modulates one or more non-inactivating conductances. Because the persistent sodium current (I_{NaP}) and I_h are both non-inactivating currents in ETCs (Liu and Shipley, 2008a), we investigated these two conductances. In the presence of the selective sodium channel blocker TTX ($1 \mu\text{M}$), DA ($20 \mu\text{M}$) induced an inward current ($8.5 \pm 1.0 \text{ pA}$, $n = 6$) that was indistinguishable from that in control ($8.1 \pm 1.5 \text{ pA}$, $n = 6$, $p > 0.05$; Fig. 4F, G). This indicates that DA does not modulate I_{NaP} in ETCs. In contrast, the selective I_h channel blocker ZD7288 ($20 \mu\text{M}$) abolished the DA-induced inward current (Fig. 4F, G). This suggests that the DA-induced inward current is attributable to enhancement of I_h . This was further confirmed by experiments (Fig. 4H, I) showing that I_h current activated by hyperpolarizing voltage steps (500 ms, 5 mV/step) was enhanced by $11.4 \pm 3.9 \text{ pA}$ ($n = 6$ cells) by DA ($20 \mu\text{M}$) over a voltage range from -85 to -50 mV . Together, these results indicate that DA acts via D_1 -like receptors to enhance I_h and produce an inward current in ETCs.

DA enhances ETC spontaneous bursting via D_1 -like receptors

The physiological hallmark of ETCs is their spontaneous burst firing behavior (Hayar et al., 2004b; Liu and Shipley, 2008a). The frequency of spontaneous bursting is voltage dependent, i.e., it increases with membrane depolarization and decreases with hyperpolarization (Hayar et al., 2004b). ETCs provide excitatory input to and receive synaptic feedback from the majority ($\sim 70\%$) of SACs (Hayar et al., 2004a; Kiyokage et al., 2010). Thus, ETC spontaneous bursting might generate DA release from SACs, and, because DA enhances I_h current in ETCs, DA feedback might increase ETC spontaneous bursting frequency. To test this, we made cell-attached recordings of ETC spontaneous bursting before and after application of DA. As shown in Figure 5, DA ($20 \mu\text{M}$) reversibly increased spontaneous burst

is abolished by the I_h -selective blocker ZD7288 but not by TTX. *** $p < 0.001$ ($n = 5$ cells, one-way ANOVA with Bonferroni's test). **H**, Population I - V curve showing that DA enhances I_h in six of six cells tested. Inset, Current traces showing ETC responses to hyperpolarizing voltage steps (5 mV/step) in the absence (black) or presence (red) of DA. **I**, Close-up of the I_h I - V curve shown in **H** in the voltage range from -60 to -50 mV .

Figure 5. DA increases spontaneous burst frequency in ETCs. **A**, Cell-attached traces showing spontaneous burst firing in an ETC before (control), during (20 μ M DA, red), and after washout of DA. **B**, Scatter plot showing that DA increases frequency of spontaneous bursting in the ETC shown in **A**. **C**, Scatter plot showing that DA increased spontaneous burst frequency is completely blocked by SCH39166 (SCH, 10 μ M). **D**, Scatter plot showing that frequency of spontaneous bursting is reversibly increased by the selective D₁-like agonist DHX (10 μ M). **E**, Population data showing that spontaneous burst frequency in 11 ETCs is reversibly enhanced by DA (20 μ M). *** p < 0.001 (n = 11 cells, one-way repeated-measure ANOVA with Bonferroni's test). **F**, **G**, Scatter plot showing that DHX (10 μ M)-induced or DA-induced increase in spontaneous burst frequency (**F**) and spikes/burst (**G**) in the absence (DA) or the presence of SCH39166 (10 μ M, SCH + DA) or (S)–(–)-sulpiride (50 μ M, Sul + DA). *** p < 0.001 (n = 7 cells, one-way ANOVA with Bonferroni's test).

frequency by $\sim 60\%$ (from 3.2 ± 0.4 to 5.1 ± 0.6 Hz; n = 11, p < 0.001); increased burst frequency was associated with decreased spikes/burst (from 4.4 ± 1.3 to 3.6 ± 1.0 ; n = 11, p < 0.001) in all cells tested (Fig. 5A,B,E). This is consistent with the previous finding that depolarization increases spontaneous bursting frequency but decreases spikes per burst (Hayar et al., 2004b). We next asked whether the DA-mediated increase in ETC spontaneous bursting is attributable to activation of D₁-like receptors. Indeed, the selective D₁-like receptor antagonist SCH39166 (10 μ M) completely blocked both the DA-induced increase in spontaneous burst frequency ($64.6 \pm 7.8\%$ increase in bursting with DA alone compared with $0.3 \pm 0.7\%$ increase when SCH39166 is present, n = 7, p < 0.001; Fig. 5C,F) and decreased spikes/burst ($20.9 \pm 1.8\%$ decrease in spiking in DA alone compared with a decrease of $0.8 \pm 0.4\%$ when SCH39166 is present, n = 7, p < 0.001; Fig. 5G). The selective D₁-like receptor agonist DHX (10 μ M) had a similar effect to DA on both spontaneous burst frequency (increase by $60.3 \pm 9.0\%$, n = 5; Fig. 5F) and spikes/burst (decrease by $19.7 \pm 1.8\%$, n = 5; Fig. 5G). These results support the conclusion that DA increases ETC spontaneous bursting via D₁-like receptors by enhancing I_h , which depolarizes the membrane.

Consistent with DA increasing ETC spontaneous bursting via D₁-like receptors, the D₁R antagonist SCH39166 (10 μ M) reduces ETC spontaneous burst frequency (Fig. 5, compare C, B). This was further confirmed in subsequent experiments (Fig. 6C) showing that SCH39166 (10 μ M) reduced ETC spontaneous burst frequency by 30.6% from 3.6 ± 0.7 Hz in NBQX and APV

to 2.5 ± 0.5 Hz with addition of SCH39166 (p < 0.001, n = 6). This suggests that spontaneous DA release from SACs feeds back onto ETCs.

GABA–DA cotransmission generates a biphasic inhibition–excitation response in ETCs

The preceding experiments show that SACs release both GABA and DA onto ETCs. GABA produces GABA_A receptor-mediated monosynaptic inhibition of ETCs and DA enhances I_h , which increases post-inhibition rebound depolarization and strengthens ETC spontaneous bursting. How do these opposing inhibitory and excitatory actions influence ETC spike output? To investigate this, we applied optical stimulation to distant glomeruli while recording from ETCs in current clamp. Light activation evoked monosynaptic IPSPs that were followed by a strong rebound spike burst. Neither component of this biphasic response was affected by AMPA and NMDA receptor blockers (Fig. 6A,B,E), indicating that iGluRs are not required for the inhibition–excitation sequence. However, the selective D₁-like receptor antagonist SCH39166 (10 μ M) significantly attenuated the post-IPSP rebound spiking from 185.2 ± 9.5 to $153.3 \pm 8.8\%$ of baseline spontaneous burst spiking (n = 6, p < 0.01; Fig. 6B,C,E). D₁-like receptor block also increased the evoked IPSP duration (58.4 ± 4.3 to 74.4 ± 5.7 ms; p < 0.01) and decay time constant (32.9 ± 3.7 to 44.1 ± 5.4 ms; p < 0.01) in six of six cells tested (Fig. 6D–H). These changes in IPSP kinetics were not associated with any significant change in IPSP amplitude (Fig. 6D).

Figure 6. Optical stimulation of SACs produces a D_1 receptor-mediated enhancement of rebound excitation in interglomerular ETCs. **A–C**, Top, Twenty current-clamp traces showing responses of the same ETC to optical stimulation of distant glomeruli in normal ACSF (**A**), ACSF containing $10 \mu\text{M}$ NBQX + $50 \mu\text{M}$ APV (**B**), and ACSF containing NBQX + APV plus $10 \mu\text{M}$ SCH39166 (**C**). Middle, Raster plots showing spike distribution for 20 individual traces in corresponding conditions. Blue vertical lines indicate the blue laser light exposure. Bottom, Spike histogram averaged from six ETCs (20 traces/cell) showing the rebound spikes to optical stimulation of distant glomeruli in corresponding conditions shown in the top. **D**, Close-up IPSPs evoked by optical stimulation of distant glomeruli within the purple rectangles shown in the corresponding top panels. Thick traces represent the average from 20 traces (gray). **E–H**, Population data showing the effect of NBQX + APV and NBQX + APV plus SCH on the optically evoked rebound spiking (**E**), spontaneous burst frequency (**F**), duration (**G**), and decay (**H**) of the optical stimulation-evoked IPSP in six ETCs. *** $p < 0.001$ ($n = 6$ cells, one-way repeated-measure ANOVA with Bonferroni's test).

These results show that DA– D_1 R enhancement of I_h strengthens the rebound spike burst that follows the initial GABAergic inhibition. Together, these findings demonstrate that GABA and DA cotransmission from SACs generates a temporally biphasic inhibition-to-excitation response in ETCs of neighboring glomeruli.

Discussion

We provide evidence that SACs release both GABA and DA to produce a biphasic inhibition–excitation response in ETCs in neighboring glomeruli. GABA causes fast inhibition via ionotropic $GABA_A$ receptors, whereas DA generates a slower excitatory response via metabotropic D_1 -like receptors. These opposing actions are orchestrated by I_h , a prominent hyperpolarization-activated, cyclic nucleotide-gated conductance in ETCs. GABA-mediated hyperpolarization engages I_h , which repolarizes the membrane toward the threshold for spiking; DA enhances I_h , further strengthening the rebound spike burst. Thus, two transmitters acting via the same intrinsic current produce a biphasic inhibition–excitation response in ETCs, neurons that play a key role in gating the glomerulus input–output function.

GABA and DA release from SACs to ETCs

DA is released from the terminals of VTA neurons projecting to the nucleus accumbens in the midbrain; VTA neuron synapses corelease the excitatory transmitter glutamate in addition to DA (Chuhma et al., 2004, 2009; Hnasko et al., 2010; Stuber et al., 2010; Tecuapetla et al., 2010; Yamaguchi et al., 2011). Recent studies showed that DA terminals in the striatum can corelease DA, GABA, and glutamate (Tritsch et al., 2012), although when iGluRs and $GABA_A$ receptors were blocked, postsynaptic DA responses were not detected, presumably because of their small current size.

Olfactory bulb SACs coexpress markers for GABA and DA (Hökfelt et al., 1975; Baker et al., 1983; Kosaka et al., 1985;

Goheen Robillard et al., 1997; Kosaka and Kosaka, 2008; Maher and Westbrook, 2008; Kiyokage et al., 2010), and SACs synaptically target multiple glomeruli forming the IGC (Aungst et al., 2003; Kosaka and Kosaka, 2008; Kiyokage et al., 2010). Although the coexistence of markers for GABA and DA in SACs is well established, there is little evidence that these two transmitters are coreleased. The finding that direct activation of TH^+ cells produced $GABA_A$ -mediated self-inhibition and DA– D_2 R-mediated presynaptic inhibition in the GL is consistent with this notion (Maher and Westbrook, 2008). The present experiments provide the first evidence that SACs release both GABA and DA onto ETCs. Optical activation of $ChR2^+$ SACs evokes $GABA_A$ -mediated IPSCs with a latency of ~ 2.2 ms, consistent with monosynaptic input. The IPSC is followed by an excitatory response that persists when the IPSC is blocked by GBZ but is blocked by D_1 R antagonists. Thus, in contrast to the striatum, in which direct postsynaptic DA responses were not seen, activation of SACs generates a clear DA response in ETCs.

SACs are generated throughout adult life, and 97% coexpress TH and GAD67 (Kiyokage et al., 2010). As they mature, TH promoter activity and mRNA expression occur in advance of TH protein (Baker et al., 1983; Baker et al., 2001). Thus, a proportion lack TH protein but express TH–Cre and can transform the AAV– $ChR2$ construct. Of the glomerular neurons expressing $ChR2$, 91% stain for TH protein, a ratio similar to that of TH promoter-driven transgenes to TH protein (Baker et al., 1983, 2001). Conceivably, the few $ChR2^+$ TH-negative neurons may express other transmitters, but the finding that optically evoked SAC $\rightarrow$ ETC responses are unaltered by iGluR blockers excludes glutamate.

Together, the most parsimonious interpretation of the present findings is that glomerular SACs corelease or cotransmit GABA and DA onto ETCs. Corelease implies that a neuron re-

leases two transmitters from the same synaptic vesicle; alternatively, the two transmitters may be released from different vesicles in the same neuron—cotransmission. Compelling evidence for GABA–DA corelease in the striatum was demonstrated recently by showing that GABA and DA are sequestered by a vesicular monoamine transporter (VMAT2) into the same vesicles in DA terminals (Tritsch et al., 2012). Additional compelling evidence for DA–GABA corelease has been documented in retina (Hirasawa et al., 2012). Activation of single dissociated DAergic amacrine cells generated simultaneous DA–GABA release events and EM immunostaining showed that some secretory organelles contained both VMAT2 and vesicular GABA transporter (VGAT). It is not known whether DA and GABA are contained in the same or different vesicles in SAC terminals. Future experiments testing SAC → ETCs response in mice lacking specific vesicular transporters and/or immunostaining for VMAT and VGAT might shed light on this. Dual patch-clamp experiments in which single identified SACs are stimulated while recording ETCs might provide more direct evidence for corelease. However, single SACs make sparse contacts with multiple neighboring glomeruli (Kiyokage et al., 2010), suggesting that postsynaptic impact depends on convergent inputs from multiple SACs; thus, activation of single SACs might not evoke consistently detectable responses in ETCs.

GABA and DA acting via I_h generate an inhibition–excitation response sequence

SAC release of GABA and DA evokes a biphasic inhibition–excitation response in ETCs via GABA_ARs and G-protein-coupled D₁-like receptors. How are these opposing synaptic actions orchestrated to shape ETC output? ETCs express a strong I_h conductance, which is partially active at resting membrane potential (Hayar et al., 2004b; Liu and Shipley, 2008a). I_h is strongly activated by IPSPs and plays a major role in post-inhibitory rebound spike bursting (Liu and Shipley, 2008b). We showed that both endogenous and exogenous DA activates D₁Rs, which enhance I_h to excite ETCs. D₁R-like enhancement of I_h occurs in rat neocortical interneurons (Wu and Hablitz, 2005) and retinal ganglion cells (Chen and Yang, 2007). Thus, DA modulation of neuron excitability via I_h is not unique to ETCs.

SAC release of GABA activates GABA_A receptors and produces IPSPs and inhibits spiking in ETCs. The hyperpolarization activates I_h , a depolarizing current that drives the membrane to more positive potentials activating I_{NAP} and $I_{T/L}$. When I_h is blocked, there is less activation of these depolarizing conductances that contribute to spike bursts (Liu and Shipley, 2008a). SAC DA release enhances I_h , increasing depolarization and rebound spiking. GABA_ARs mediate fast membrane changes, whereas G-protein-coupled D₁Rs involve slower multistage signaling cascades; thus, there is a time lag between the actions of the two transmitters. As a result, the ETC is initially inhibited and then switched to a DA-enhanced, I_h -dependent rebound excitation.

Circuit implications

Electrical stimulation of the IGC causes GABA_AR-mediated inhibition of ON-evoked long-lasting depolarizations (LLDs) in mitral cells (Aungst et al., 2003; Shirley et al., 2010). SACs generate a biphasic inhibition–excitation response in ETCs, which monosynaptically excite M/TCs (Hayar et al., 2004a; De Saint Jan et al., 2009; Gire et al., 2012). How might the biphasic action of SACs impact the ETC → M/TC pathway to alter glomerular output? ON input to a glomerulus would increase activity of its SACs.

This should cause transient inhibition followed by rebound excitation of ETCs of neighboring glomeruli. While inhibited, ETCs should be less sensitive to ON input; this should reduce ON → ETC → M/TC excitation. ETCs also give strong monosynaptic input to local GABAergic PGCs, which provide fast feedforward inhibition of M/TCs (Shao et al., 2012). SAC inhibition of ETCs should decrease excitatory drive on PGCs reducing inhibitory inputs to M/TC. Thus, SAC inhibition of ETCs might reduce excitatory drive on M/TCs while simultaneously reducing feedforward inhibition. If M/TCs receive the majority of their ON input via ETCs, as suggested by some (Gire et al., 2012), then SAC → ETC inhibition might render M/TCs less sensitive to sensory input. Alternatively, if M/TCs receive some direct ON input, as suggested by others (Najac et al., 2011; Shao et al., 2012), then SAC → ETC inhibition should reduce excitatory drive of PGCs and decrease inhibition of M/TCs. This might increase M/TC sensitivity to direct ON inputs. If M/TCs are excited by both direct ON input and indirect input from ETCs, then SAC inhibition of ETCs could transiently shift the effectiveness of the two sources of sensory drive on glomerular output neurons. The ensuing period of post-inhibitory rebound excitation should increase the excitatory drive of ETCs on M/TCs, but this might be balanced by increased ETC → PGC → M/TC feedforward inhibition.

The balance between these excitatory and inhibitory actions is difficult to predict absent experimental evidence, but it seems likely that the timing of the SAC → ETC inhibition–excitation sequence will strongly influence the dynamics of sensory processing in neighboring glomeruli. Electrical stimulation of the isolated IGC inhibits ON-evoked LLDs in M/TCs, but the temporal dynamics of this inhibition have not been established (Aungst et al., 2003; Shirley et al., 2010). Finally, the impact of SACs on neighboring M/TCs may not be limited to their actions via ETCs. SACs may directly synapse onto M/TCs; preliminary experiments suggest that this is likely. Thus, SACs may directly and indirectly gate sensory coding in M/TCs.

Rodents sample odors by repetitive sniffing ranging from 1 to 10 Hz. ETC spike bursts are entrained by repetitive ON inputs over this same frequency range; higher-frequency ON inputs entrain more ETCs (Hayar et al., 2004b). Because ETCs provide most of the excitatory input to SACs, higher-frequency sniffing may entrain more SACs (Wachowiak and Shipley, 2006). Our results show that ETCs are inhibited for ~60 ms after activation of SACs, after which they generate rebound bursting for ~120 ms. Thus, the “duty cycle” is ~5 Hz, and rebound excitation lasts twice as long as the initial inhibition. Interestingly, animals exhibit sniff rates in this frequency range as they encounter novel odors (Verhagen et al., 2007; Wesson et al., 2008). Sniffing near 5 Hz might bring odor sampling in register with the SAC → ETC inhibition–excitation duty cycle to optimize interglomerular regulation of sensory signals.

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